Baseline Susceptibility of *Bemisia tabaci* B Biotype (Hemiptera: Aleyrodidae) Populations from California and Arizona to Spiromesifen

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ABSTRACT Susceptibility to spiromesifen, a tetronic acid derivative, was determined for three imidacloprid-resistant strains and 12 geographically discrete natural populations of *Bemisia tabaci* (Gennadius) (=*Bemisia argentifolii* Bellows & Perring) (Hemiptera: Aleyrodidae) from California and Arizona by laboratory bioassays. Newly emerged first instars were sprayed with aqueous serial dilutions of spiromesifen and evaluated for toxicity to establish baseline susceptibility data. Interpopulation variability in susceptibility to spiromesifen was observed among the natural populations of whiteflies up to 29-fold; however, there was only 30-fold difference in susceptibility among natural and resistant populations tested. In general, spiromesifen was quite toxic to first instars across most of their geographic range, with LC_{50} values ranging from 0.210 to 6.08 μg (AI)/ml. The magnitude of variation was smaller among the three-resistant strains. These results suggest that the observed variability reflect natural variation in spiromesifen susceptibility among all the test populations, possibly due to previous exposure to insecticides at each location. The effectiveness of spiromesifen also was evaluated against all immature stages of whiteflies from three field and two resistant strains. Spiromesifen was significantly more active against early instars of whiteflies based on lower LC_{50} values recorded compared with the fourth instars. Spiromesifen was effective against the resistant strains including a Q-biotype of *B. tabaci* from Spain, which is highly resistant to neonicotinoids. Results of this study indicate absence of cross-resistance between spiromesifen and more commonly used neonicotinoids. Our findings suggest that spiromesifen should be considered an ideal candidate for whitefly resistance management programs in rotation with other effective chemistries.

KEY WORDS Oberon, *Bemisia argentifolii*, Q Biotype, whitefly immature stages, imidacloprid-resistant whiteflies

In the American southwest, *Bemisia tabaci* (Gennadius) B biotype (=*Bemisia argentifolii* Bellows & Perring) (Hemiptera: Aleyrodidae) has been a principal pest of vegetables, ornamentals, and field crops for many years, and it is considered to be one of the most serious agricultural and horticultural pests worldwide. Lost farm revenues in excess of hundreds of millions of dollars resulted due to destructive whitefly outbreaks in early 1990s in Arizona, California, and Texas (Perring et al. 1993). Relatively ineffective broad-spectrum insecticide applications were the mainstay of whitefly control at that time, but often were further diminished by serious resistance problems (Prabhaker et al. 1992, Cahill et al. 1995, Dennehy and Williams 1997). The commercial introduction of imidacloprid, a neonicotinoid, beginning in Arizona in 1993 represented a novel mode of action and quickly became the foundation of insecticide management program in mixed cropping systems. Newer compounds including the insect growth regulators (IGRs), buprofezin and pyriproxyfen, and later generation neonicotinoids fortified chemical control of *B. tabaci*, but not without resistance risks, especially to the neonicotinoids due to their proliferation across crop commodities (Palumbo et al. 2003). Wide variability in bioassay responses to imidacloprid in Arizona and California *B. tabaci* populations (Dennehy et al. 1999, Prabhaker et al. 2005) has indicated the potential for neonicotinoid resistance, a problem already observed in *B. tabaci* in other regions of the world (Elbert and Nauen 2000, Nauen et al. 2002, Horowitz et al. 2004). New modes of action could provide relief from the high selection pressure placed upon imidacloprid and other neonicotinoids through their intensive use against *B. tabaci* in southwestern U.S. agriculture.

In addition to the use of neonicotinoids and IGRs for managing whiteflies, several options in chemical

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control are available through development of integrated pest and resistance management programs that include the use of safer, more selective insecticides with novel modes of action. Some of the newer insecticides, including sodium channel blockers and tetronic acid derivatives, are more selective, and they are reported to be compatible with the use of many kinds of natural enemies (Nauen et al. 2003, Elbert et al. 2005). Spiromesifen is a member of the spirocyclic tetronic acid derivatives, a recently introduced class of selective chemistry with insecticidal and acaricidal activity (Bretschneider et al. 2003; Nauen et al. 2003, 2005). Spiromesifen has properties that are similar to IGRs that affect developing life stages without any signs of neurotoxic activity. Spiromesifen acts as an inhibitor of lipid biosynthesis and interferes with development of the egg and immature stages and reduces adult female fecundity (Bretschneider et al. 2003). Foliar sprays of spiromesifen have been effective against whiteflies in cotton (*Gossypium hirsutum* L.), vegetables, melons, and ornamentals (Palumbo 2004, Elbert et al. 2005). Spiromesifen is unrelated to neonicotinioids, making it a good rotational candidate in a resistance management program for whiteflies.

Resistance monitoring studies determine pest population responses to a particular compound, ideally before commercial use begins in an area. Establishment of baseline susceptibility levels to spiromesifen in whitefly populations is a critical step in the development of resistance management strategies. Baseline susceptibility data provide a reference for comparing shifts in susceptibility to spiromesifen after widespread use and will enable detection of early stages of resistance development. Monitoring is also important to show that populations of a pest from various geographic regions can show natural variations in responses to the same compound which may be unrelated to resistance problems. Therefore, it is critical to establish the susceptibility levels of various populations at the outset even before the widespread use of spiromesifen. Additionally, continuous monitoring of whitefly populations to document shifts in sensitivity after the commercial introduction of spiromesifen will aid in implementing resistance management strategies before control failures occur.

This article describes monitoring results of a number of whitefly populations from California and Arizona to spiromesifen through bioassays in the laboratory to determine the baseline susceptibility data. Bioassay tests conducted in the laboratory under controlled conditions also can make possible comparison of the relative toxicities of various compounds that have been similarly investigated. In addition to evaluating responses of natural whitefly populations to spiromesifen, we also determined the susceptibility of three imidacloprid-resistant whitefly strains to spiromesifen to assess any potential for cross-resistance between spiromesifen and imidacloprid. Knowledge of cross-resistance patterns of neonicotinoids and other compounds is critical for the development of a successful resistance management program for whiteflies. The current study also compared the efficacy of spiromesifen between different immature stages of five strains of *B. tabaci.*

Table 1. Locations, crops, and year of collection of whitefly populations from Arizona and California in 2005 and 2006

Collection/state	Location	Yr of collection	Crop
California	Brawley	Sept. 2005	Cotton
	El Centro	Aug. 2005	Cotton
	Holtville	June 2005	Melon
	El Centro	Nov. 2006	Broccoli
	Brawley	June 2006	Melon
	Holtville	April 2006	Melon
Arizona	Maricopa	Sept. 2005	Cotton
	Maricopa	Oct. 2005	Weeds
	Yuma	June 2005	Melon
	Maricopa	Nov. 2006	Broccoli
	Phoenix	May 2006	Ornamentals
	Yuma	June 2006	Melon

Materials and Methods

Field Populations and Resistant Strains

Field Populations: *B. tabaci* B Biotype (=*B. argentifolii*). Twelve field populations of *B. tabaci* from multiple sites in California and Arizona were collected in 2005 and 2006 and tested for toxicity responses to spiromesifen to establish baseline susceptibility data. Monitoring site details are provided in Table 1. The majority of the adult whitefly collections were made from cotton, broccoli, melons, ornamentals, and weeds in Imperial Valley, CA, Maricopa, AZ, and Yuma, AZ, over a 12-mo period from June 2005 to June 2006. Adult whiteflies were collected directly from the leaves of various field crops with a battery-operated vacuum sampler and transported to the laboratory confined in transfer cages containing cotton seedlings. Subsequently, insects were transferred to colony cages (152 by 101 by 116 cm) with clean cotton plants to allow oviposition for obtaining immatures for testing. Tests with spiromesifen were conducted against the immature stages to determine susceptibility.

Imidacloprid-Resistant Strain (IM-R Strain), *B. tabaci* B Biotype (=*B. argentifolii*). A resistant strain of *B. tabaci* was developed by selectively breeding for imidacloprid resistance (Prabhaker et al. 1997). For selection of imidacloprid resistance, whitefly pupae on melon leaves were originally collected in May 1993 from imidacloprid-treated melon fields in Imperial Valley, CA. Approximately 12,000–15,000 adult whiteflies were collected upon emergence and caged over a 2-wk period on young cotton plants treated systemically with imidacloprid to initiate an imidacloprid-resistant strain. This strain has been maintained in the laboratory under selection on cotton for a number of years. Fresh whitefly collections made from Imperial Valley, CA, were infused into this colony at various times over the last 5 yr to enlarge the gene pool to avoid bottlenecks. Resistance to imidacloprid during this study period was ≈100-fold.

Guatemalan-Resistant Strain (Guat-R), *B. tabaci* B Biotype. A strain of whiteflies from Guatemala that were resistant to imidacloprid was established at the USDA facility in Phoenix, AZ, in 2001 (Prabhaker et al. 2005). Melon leaves infested with whitefly immatures were collected from imidacloprid-treated melon fields and received from Guatemala at various times in 2000 and 2001 for resistance evaluation to imidacloprid. The melon leaves infested with the immatures were maintained in wooden cages (152 by 101 by 116 cm) to allow for emergence. Emerged whiteflies settled on melon plants in the cage and were later used for selection and testing. Once the strain was well established on melons after approximately two to three generations, cotton plants were used for further maintenance and tests. This strain has been under selection with systemic applications of imidacloprid at various times to maintain resistance to this compound. Bioassays with spiromesifen were conducted on this strain to assess for cross-resistance to imidacloprid.

Spanish-Resistant Strain (SQ-R), *B. tabaci* Q Biotype. The Q biotype of *B. tabaci* Spanish populations was described in 1997 (Guirao et al. 1997). The SQ-R strain was collected on capsicum in Almeria, Spain, in 2003 and was transported back to USA in containers with fresh leaves to the quarantine laboratory in Riverside, CA. The Q strain was established on cotton for our studies. Evaluations with four neonicotinoids confirmed that the SQ-R strain exhibited high cross-resistance levels to four neonicotinoids, acetamiprid, dinotefuran, imidacloprid and thiamethoxam (Prabhaker et al. 2005). It is also well documented that whiteflies from Almeria, Spain, were highly resistant to imidacloprid and most of the conventional insecticides (Elbert and Nauen 2000) that have been used routinely in the area's vegetable crops.

Insecticide

A formulated grade of spiromesifen (Oberon 2 SC, 23.1% [AI]), provided by Bayer CropScience, (Kansas City, MO) was used for bioassays to establish baseline susceptibility in whiteflies. Stock solutions and serial dilutions of the formulated product were freshly made with deionized water on the day of tests for use in bioassays. Five concentrations of spiromesifen were used to determine the LC_{50} expressed as micrograms (AI) per milliliter.

Bioassay Technique

Baseline susceptibility of each population to spiromesifen was determined by a leaf spray technique to measure concentration–mortality responses of selected immature stages. 'Deltapine 5415' cotton plants in the two true-leaf stage were used for tests. Approximately 40 adult whiteflies from the field or the resistant colonies were caged on individual cotton leaves in small clip cages for a 24-h oviposition period to allow synchronization of each developing stage. After oviposition, adults were removed from the infested leaves, and the number of eggs laid on each leaf was recorded. Test plants were maintained at 27 ± 2°C, 80 ± 5% RH, and a photoperiod of 14:10 (L:D) h in all cases, with the exception of the SQ-R strain, which was maintained under a photoperiod of 12:12 (L:D) h in the Quarantine facility. Infested plants were used at appropriate times for tests against a specific immature stage as described below.

To assess responses of various whiteflies to spiromesifen for monitoring purposes, treatment was directed against first instars. The synchronized cohort of eggs was provided a 7-d incubation period until hatch, after which crawlers were allowed 24 h to settle. Leaves infested with settled first instars were sprayed directly on the abaxial side until runoff (equal to six sprays) by aqueous serial dilutions of spiromesifen by using a 118 ml (4-oz). plastic bottle equipped with a fine mist sprayer. Control plants were sprayed with water. Treated cotton plants were maintained in insect free greenhouses for at least 7 d. Mortality of first instars was recorded 48 h posttreatment. Subsequently, mortality checks were made after 96 h in case some of the first instars survived to the next stage. Five replicates were included per concentration for each test. The criterion for mortality of first instars was based on the dryness of individuals and whether they separated from the leaf when lifted with a needle. Mortality of only first instars was used to calculate probit parameters for establishment of baseline data.

In addition to treatment of first instars for baseline toxicity tests, select field populations, El Centro, Holtville, and Yuma, and two resistant strains of whiteflies, IM-R and SQ-R, were tested for efficacy of spiromesifen against each immature life stage. Egg-infested cotton plants as described above were maintained in insect-free greenhouses to allow for development of different immature stages before tests. Each immature stage was treated by spraying the abaxial side of attached infested cotton leaves until runoff with aqueous serial dilutions of spiromesifen. The first instars were treated on day 8 postoviposition, second instars on day 10 postoviposition, third instars were treated after 13 d, and fourth instars were treated on day 16 after oviposition. The control leaves were sprayed with water alone. Mortality was checked after 48 h posttreatment for each stage. The same criterion for mortality of immatures described above was followed for these tests also.

Data Analysis

Estimations of probit parameters of the concentration–mortality responses of various populations of whiteflies from the leaf spray bioassays were calculated by POLO (Russell et al. 1977). The parameters included calculations of LC_{50} values and their corresponding 95% confidence limits (CL), slopes of regression, and the g value. The POLO probit analysis model generates the g factor to indicate the level of fit for analyzed data which should be <0.5. For comparison of mortality responses between populations, two LC_{50} values were considered significantly different if

Table 2. Baseline susceptibility expressed as LC_{50} values of various natural and resistant populations of *B. tabaci* (=*B. argentifolii*) from Arizona, California, and Spain (Q-type) to spiromesifen

Location/state	Location/field	Collection yr	*n*	Slope ± SE	LC_{50} (μg[AI]/ml) (95% CL)[a]	χ^2 (df)	g (0.95)[b]
California	Brawley	2005	865	1.2 ± 0.05	1.16 (0.743–1.82)b	6.7 (4)	0.26
	El Centro	2005	1278	1.3 ± 0.10	0.802 (0.466–1.58)ab	8.4 (4)	0.39
	Holtville	2005	907	1.3 ± 0.08	5.40 (2.29–9.02)c	8.8 (4)	0.13
	El Centro	2006	838	1.9 ± 0.11	3.45 (2.18–6.58)c	7.1 (4)	0.42
	Brawley	2006	957	1.9 ± 0.21	0.880 (0.494–1.09)b	5.9 (4)	0.10
	Holtville	2006	884	1.9 ± 0.18	0.871 (0.485–1.36)b	5.1 (4)	0.22
Arizona	Maricopa	2005	892	1.3 ± 0.11	4.40 (2.22–7.54)c	7.2 (4)	0.16
	Maricopa	2005	1142	1.6 ± 0.06	0.210 (0.092–0.474)a	7.0 (4)	0.32
	Yuma	2005	962	1.1 ± 0.05	1.33 (0.759–2.11)b	5.7 (4)	0.09
	Maricopa	2006	886	1.3 ± 0.13	6.08 (3.45–10.06)c	6.2 (4)	0.21
	Phoenix	2006	1027	1.2 ± 0.10	0.434 (0.188–0.858)ab	6.4 (4)	0.08
	Yuma	2006	998	1.5 ± 0.07	0.950 (0.474–1.82)ab	5.2 (4)	0.23
Resistant strains	IM-R	2005	1217	2.5 ± 0.21	5.16 (2.91–8.45)c	6.5 (4)	0.25
	GU-R	2005	815	2.0 ± 0.09	6.29 (3.10–10.28)c	7.1 (4)	0.31
	SQ-R	2005	859	2.1 ± 0.11	2.62 (1.98–5.48)c	7.3 (4)	0.27

[a] LC_{50} values followed by the same letter are not significantly different based on overlap of 95% CLs across whitefly populations.
[b] 'Index of significance for potency estimation 'g' will be substantially smaller than 1.0 and seldom >0.4.' (Finney, 'Probit Analysis' 1971, p. 79).

there was no overlap between their corresponding 95% CL.

Results

Field Populations. Of the 12 populations collected across California and Arizona in 2005–2006, susceptibility to spiromesifen varied significantly, even though spiromesifen had not been used in locations of whitefly collections. The LC_{50} values ranged from 0.210 to 6.08 μg (AI)/ml spiromesifen, a 29-fold variability difference between the least and most sensitive populations (Table 2). Two populations tested from Imperial Valley (Holtville and El Centro) and two from Maricopa, AZ, had significantly higher LC_{50} values—3.45, 5.40, 4.40, and 6.08 μg (AI)/ml, respectively—than all remaining field populations. At the low end of the response spectrum, LC_{50} values ranged from 0.210 to 1.33 μg (AI)/ml, a variability difference of only 6.3-fold. The population from Maricopa collected on weeds was the most sensitive to spiromesifen based on the lowest LC_{50} value, but it was not significantly different from El Centro or Yuma populations (based on overlapping confidence limits). Populations from Brawley and Holtville were intermediate in their responses to spiromesifen. In general, the LC_{50} values of spiromesifen for most populations from both California and Arizona ranged around an average response of 1 μg (AI)/ml, suggesting that spiromesifen was effective against these insects.

Significant differences in susceptibility of first instars to spiromesifen were identified in two of the six populations tested from California (Holtville and El Centro). The LC_{50} values for California populations ranged from 0.802 to 5.40 μg (AI)/ml (7-fold), even though these populations were within 30 km of one another (Table 2). Also significant variation in susceptibility to spiromesifen existed among populations within the same area from year to year. For example, El Centro populations were 4–fold less sensitive from 2005 to 2006. The two whitefly collections from Holtville showed a significant susceptibility difference of 6-fold as indicated by nonoverlapping confidence limits from 2005 to 2006. A similar range of responses (0.210–6.08 μg [AI]/ml) was observed in the three populations collected from the central region of Arizona represented by Maricopa collections. By contrast, bioassay test responses of whiteflies were more consistent within each area of Yuma (LC_{50} values from 0.950 to 1.33 μg [AI]/ml). Whiteflies collected on ornamentals in Phoenix were highly susceptible to spiromesifen (LC_{50} = 0.434 μg [AI]/ml) compared with some of the Maricopa populations.

Resistant Strains. Bioassay tests indicated that toxicity responses of the three imidacloprid-resistant strains, IM-R, GU-R, and SQ-R, to spiromesifen were around the baseline range of the four field populations that exhibited higher LC_{50} values (3.45–6.08 μg [AI]/ml). The LC_{50} values of resistant strains ranged from 2.62 to 6.29 μg (AI)/ml (Table 2). The variation observed between the three resistant strains was minimum and not significant based on overlapping confidence limits, indicating that test responses were more consistent between the resistant strains than those of the field populations. However, compared with some of the field populations, there were significant differences in toxicity to spiromesifen between the imidacloprid-resistant strains and the field populations, the difference ranging up to 30-fold. Despite the absence of any previous exposure to spiromesifen, the three imidacloprid-resistant strains showed LC_{50} responses to spiromesifen that were higher than some field populations but at the same time were not significantly different from four other field populations with higher LC_{50} values. These results indicate the absence of any cross-resistance between spiromesifen and imidacloprid.

The slopes determined by probit analysis for the field populations were generally low ranging from 1.1 to 1.9. These low values are not unexpected, because

Table 3. Effect of spiromesifen on four immature stages of select field and imidacloprid-resistant whitefly populations

Location/insect strain	Immature stages[a]	*n*	Slope ± SE	LC_{50} (μ g[AI]/ml) (95% CL)[b]	χ^2 (df)	g (0.95)[c]
El Centro (2005)	First instar[a]	1278	1.3 ± 0.10	0.802 (0.466–1.58)a [a][d]	8.4 (4)	0.39
	Second instar	936	1.4 ± 0.11	0.905 (0.810–2.98)a [a]	5.7 (4)	0.25
	Rhird instar	805	2.0 ± 0.19	1.91 (0.922–2.87)a [a]	7.6 (4)	0.37
	Fourth instar	836	2.5 ± 0.26	6.34 (5.17–8.89)b [a]	6.9 (4)	0.32
Holtville (2006)	First instar[a]	884	1.9 ± 0.18	0.871 (0.485–1.36)a [a]	5.1 (4)	0.22
	Second instar	963	1.3 ± 0.13	1.01 (0.904–3.11)a [a]	4.6 (4)	0.25
	Third instar	780	1.4 ± 0.12	1.62 (0.968–3.55)a [a]	5.1 (4)	0.03
	Fourth instar	929	1.2 ± 0.07	4.22 (3.73–8.43)b [a]	5.3 (4)	0.09
Yuma (2006)	First instar[a]	998	1.5 ± 0.07	0.950 (0.474–1.92)a [a]	5.2 (4)	0.22
	Second instar	1008	1.3 ± 0.11	0.992 (0.398–1.98)a [a]	7.3 (4)	0.33
	Third instar	881	1.7 ± 0.13	2.94 (1.16–4.08)a [a]	6.9 (4)	0.12
	Fourth instar	752	1.6 ± 0.14	6.77 (4.60–10.83)b [a]	5.1 (4)	0.03
IM-R	First instar[a]	1217	2.5 ± 0.21	5.16 (2.91–8.45)a [b]	6.5 (4)	0.25
	Second instar	797	2.7 ± 0.32	6.85 (5.73–8.14)a [b]	4.6 (4)	0.32
	Third instar	728	2.4 ± 0.25	15.79 (8.72–18.57)b [b]	6.5 (4)	0.38
	Fourth instar	784	2.6 ± 0.26	16.14 (11.94–18.95)b [b]	5.9 (4)	0.44
SQ-R	First instar[a]	859	1.9 ± 0.08	2.62 (1.98–5.48)a [b]	7.3 (4)	0.24
	Second instar	831	1.2 ± 0.06	7.16 (6.82–14.49)b [b]	5.8 (4)	0.27
	Third instar	719	1.3 ± 0.05	17.86 (10.28–22.41)bc [b]	7.7 (4)	0.40
	Fourth instar	651	1.9 ± 0.11	21.77 (14.75–26.44)c [b]	8.8 (4)	0.33

[a] Data for first instars are taken from Table 2.
[b] LC_{50} values followed by the same letter are not significantly different based on overlap of 95% CLs across stages within a population.
[c] Index of significance for potency estimation 'g' will be substantially smaller than 1.0 and seldom >0.4. (Finney, Probit Analysis 1971, p. 79).
[d] LC_{50} values followed by the same letter within brackets are not significantly different for a particular immature stage, across the five populations.

field populations not exposed to a new compound previously exhibit higher levels of heterogeneity within populations. The slope values were higher for the resistant strains (ranging from 2.0 to 2.5) indicating less heterogeneity within these strains perhaps due to selection for resistance to imidacloprid over time.

Evaluation of Spiromesifen against Four Immature Life Stages of Whiteflies. The differences in susceptibility of four immature stages of three field populations and two imidacloprid-resistant whitefly strains to spiromesifen are presented in Table 3. The overall LC_{50} values for first to fourth instars varied according to age of instar and source of insects. In general, the LC_{50} values increased with age. Based on higher LC_{50} values overall, older nymphs were less sensitive than younger nymphs, requiring higher concentrations of spiromesifen to attain equal mortality levels.

Within population comparisons showed that the LC_{50} values among the first three instars of the three field populations (El Centro, Holtville, and Yuma) were not significantly different (based on overlapping confidence limits) from one another. The fourth instars of the three field populations were significantly less sensitive (4.22–6.77 μg [AI]/ml) to spiromesifen compared with the three younger stages. Across-field comparisons of the three populations showed no significant differences in responses of each stage to spiromesifen, indicating a similar trend as within-field population responses for all four immature stages.

The responses of the four immature stages of the two resistant strains to spiromesifen showed a different trend than the field populations (Table 3). The first and second instars of the IM-R strain were significantly more sensitive to spiromesifen than the third and fourth instars. By contrast, the first instars were significantly more susceptible to spiromesifen than second, third, and fourth instars of the SQ-R strain. Also second instars of the SQ-R strain were significantly different from the fourth instars. Susceptibility comparisons of each nymphal stage across the two resistant strains to spiromesifen show no significant differences from stage to stage similar to the field populations.

The first instars were the most sensitive to spiromesifen in general, as indicated by the lowest LC_{50} values. However, the first instars of IM-R and SQ-R were significantly different from the field populations (0.802–0.950 μg [AI]/ml) in susceptibility, showing higher LC_{50} values of 5.16 and 2.62 μg (AI)/ml for IM-R and SQ-R, respectively. Significant differences were also observed in spiromesifen susceptibility across second instars of field populations (LC_{50} values ranging from 0.905 to 1.01 μg [AI]/ml) and those of the resistant strains, IM-R and SQ-R (LC_{50} values ranging from 6.85 to 7.16 μg [AI]/ml), field populations being at least 7- to 8-fold more sensitive. No significant difference in susceptibility was observed between first and second instars within each population of whiteflies (LC_{50} values ranging from 0.905 to 7.16 μg [AI]/ml) except in the case of the SQ-R strain. But there was a significant difference of 6- to 11-fold less sensitivity between third instars of resistant versus field strains. In contrast to the first instars, the fourth instars were the least sensitive to spiromesifen in both field and resistant strains (LC_{50} values ranging from 4.22 to 21.77 μg [AI]/ml). The fourth instars of the field populations were two- to five-fold significantly more sensitive (nonoverlapping confidence limits) than the resistant whiteflies. In spite of the differences in susceptibility to spiromesifen between the older

stages of field and resistant strains, spiromesifen was still toxic to the third and fourth instars of the resistant strain but at higher doses (LC_{50} values ranging from 15.79 to 21.77 μg [AI]/ml) compared with the younger instars. Also, 94% of the treated insects in these tests did not complete development to the adult stage (data not shown) indicating that spiromesifen was highly effective.

Discussion

The monitoring data show that considerable variation in susceptibility to spiromesifen existed across geographically discrete populations of *B. tabaci*. Of the 12 natural populations evaluated, four populations, two from California and two from Arizona, were the least sensitive compared with the remaining eight populations. The populations from Brawley, Holtville, Yuma, and Phoenix were generally more sensitive and consistent in their response to spiromesifen, whereas the population from Maricopa, AZ, collected on weeds was the most susceptible to spiromesifen. Interestingly, both the least and most sensitive field populations to spiromesifen were from Maricopa exhibiting a 29-fold variation between them suggesting that whitefly populations from this region were highly variable and inconsistent in their responses. There are many possible susceptibility factors that could play into a difference of this magnitude. Pesticide exposure histories, host plant differences or other environmental stressors such as temperature can influence the physiological condition of field-collected subjects represented in the bioassays. Host plants also can modify the susceptibility of herbivorous pests to insecticides (Yu 1986, Brattsen 1988), which may be related to different levels of metabolic enzymes (Ambrose and Regupathy 1992, Tan and Guo 1996). The variability also can be due to genetic differences between populations.

The current study has shown that the three imidacloprid-resistant strains were generally less sensitive to spiromesifen compared with some of the field populations. However, four of the field populations showed less sensitivity to spiromesifen similar to the three resistant strains. The four populations were collected in areas of heavy neonicotinoid use, but the slightly elevated response of these four populations along with the three imidacloprid-resistant strains does not suggest a low level of cross-resistance due to preexisting resistance to spiromesifen. Rather, the variability observed could be related to preexisting metabolic and/or excretion mechanisms selected by previous exposure to various insecticides in each geographic region over time. Our results are similar to previous studies that reported on the high performance of spiromesifen against younger stages of pyriproxyfen- and imidacloprid-resistant whiteflies (Guthrie et al. 2003), suggesting the absence of cross-resistance between pyriproxyfen, imidacloprid and spiromesifen. The similarities in responses of both natural and resistant whiteflies to spiromesifen in this study suggests that ideally, new insecticides should be evaluated against both field and resistant strains of pests to confirm their effectiveness.

Variations in susceptibility to spiromesifen were reported in an earlier study for *Bemisia* biotypes B, K and Q from different geographic regions (Nauen and Konanz 2005). The LC_{50} estimates reported for the different strains were lower (0.09–2 μg [AI])/ml) than the insects from California and Arizona, with a variability factor up to 20-fold for both susceptible and resistant strains. Our studies estimated toxic values that were higher than 2 μg (AI)/ml in some of the field populations with a higher variation (29-fold) between populations. Additionally, our results estimated the LC_{50} values of spiromesifen to be higher for the resistant strains compared with the previous study by Nauen and Konanz (2005). But both studies showed variability in responses of whiteflies to spiromesifen regardless of known resistance factors present in some strains, suggesting that the existing variability was natural and not due to cross-resistance between insecticides. The variability differences may be caused by the genetic background of the test populations which have different exposure history to insecticides. There also may be differences due to variability in biological assays. But more importantly, the known mode of action for spiromesifen is unique—inhibition of lipid biosynthesis—which excludes metabolic or target site interactions, suggesting lack of cross-resistance between insecticides.

The lack of significant differences in relative susceptibilities between the younger life stages of field populations can be attributed to the heterogeneous responses observed. It was not unexpected that the first instars were the most susceptible to spiromesifen followed by second instars. Results consistently showed the fourth instars to be the least sensitive of all whitefly immature life stages regardless of whether the insects were natural or resistant. Mortality was highest in the first immature stage of *Homalodisca vitripennis* (Germar) (formerly known as *H. coagulata* Say) by buprofezin compared with the older immature stages (Prabhaker and Toscano 2007). Our monitoring results are based on treatment of first instars compared with results obtained by testing second instars in the study reported by Nauen and Konanz (2005). It was necessary to test all developing life stages in bioassays for gauging the best effectiveness of spiromesifen against the most susceptible stage, which seemed to be the first instar in our results, although there was no significant difference in toxicity to the second instars in field populations. Therefore, the use of either first or second instars will be suitable for future monitoring purposes to assess shifts in sensitivity to spiromesifen. Targeting insecticide applications against the most susceptible stage may be economically viable to control in terms of using lesser amounts of insecticide applied. Harris (1972) described the differences in susceptibility of developmental stages of insects to insecticides. Other studies also demonstrated that the early instars of different species were more susceptible than the older instars to various insecticides (Stuijfzand et al. 2000, Wang et al.

2003, Prabhaker et al. 2006, Prabhaker and Toscano, 2007). Results on the differential susceptibility reported in this study may be attributable to size, because older instars increase in body weight compared with the younger instars; therefore, they would need a higher quantity of toxin to obtain the same level of mortality. Physiological differences related to defense mechanisms between the younger and older larvae may be another reason. The results of this study showing the differences in sensitivities between developmental stages of whiteflies to spiromesifen can aid in the selection of suitable application timings to fully exploit the mode of action of this new insecticide against the most susceptible immature stage.

The discovery of new reduced-risk insecticides such as IGRs, neonicotinoids, spinosad, and tetronic acid derivatives has provided crop protection with new classes of chemistries as alternatives to conventional insecticides. Most IGRs are active against the immature stages of a number of insect species (Dhadialla et al. 1998) by disrupting the molting process or cuticle formation (Tasei 2001). Generally, they are slow-acting compounds against specific stages of target pests (Casida and Quistad 1998). Spiromesifen has properties similar to IGRs in that it disrupts the normal processes of growth and development by inhibiting lipid biosynthesis, which leads to dehydration and death of the immatures. Spiromesifen showed exceptional activity against immature whiteflies under field conditions such as in spring melons, suggesting that this compound can be an important alternative for whitefly management for melon growers in Yuma, AZ (Palumbo 2004), where neonicotinoids and IGRs have been used for more than a decade. Spiromesifen also showed excellent activity against greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Bi and Toscano 2007), young *B. tabaci* whitefly nymphs and mites in vegetable and field crops, and also against the potato psyllid, *Paratrioza cockerelli* (Sulc), in tomatoes, peppers, and potatoes (Elbert et al. 2005). This information on the effectiveness of spiromesifen under field conditions is important and suggests that field insects that may be resistant to one or more commonly used insecticides are not showing cross-resistance to spiromesifen, and it is possible to incorporate this compound well in whitefly IPM programs.

The data in this survey provide useful information on natural variation in susceptibility to spiromesifen in populations of *B. tabaci* from California and Arizona before widespread use of the compound. These data may be used as baselines for monitoring changes in susceptibility to spiromesifen in these regions and perhaps other geographic regions, which is essential to its long-term sustainability. These results also indicate the sensitivity of the leaf spray bioassay technique, which can be used in a continuing monitoring study of whiteflies to check development of tolerance to spiromesifen over time. The current study shows the importance of sampling as many fields as possible to establish any variations in susceptibility of whiteflies to spiromesifen in different geographic locations that could be due to factors other than resistance. The information presented in this article gives a quantitative basis for successful use of spiromesifen in the management of whiteflies by timing applications to target the most susceptible life stage of whiteflies. Based on these results, spiromesifen seems to be an important alternative insecticide that can be a valuable addition to existing arsenal of chemistries used to manage whiteflies in California and Arizona.

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